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DISEASE DYNAMICS AND CROSS-TRANSMISSION RISKS IN CULTURED AND WILD BARRAMUNDI (*Lates calcarifer*) IN INNER AMBON BAY, INDONESIA

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ABSTRACT

This study aimed to evaluate the potential infection of bacteria *Vibrio* spp. and parasites in farmed barramundi (*Lates calcarifer*) and wild fish inhabiting Inner Ambon Bay. The research was conducted over a five-month period (August–December 2024) using a purposive sampling, to monthly collect and analyse two barramundi populations. Bacteria were isolated from liver and kidney tissues of the collected fish using sea water complate (SWC) agar and tiosulfate citrate bile-salt sucrose (TCBS) agar and identified with the Analytical Profile Index (API) 20 Non-Enteric (NE) kit. Parasitic identification was performed microscopically on gills, skin, and intestinal samples. The results revealed that *Vibrio* spp. were detected at relatively high abundance, dominated by *V. harveyi*, *V. alginolyticus*, and *V. vulnificus*. Identified parasites included *Trichodina* sp., *Cryptocaryon irritans*, *Benedenia* sp., and nematodes. Parasite infection prevalence in cultured barramundi ranged from 40% to 70%, whereas in wild fish it reached 60%. These findings strongly indicate the potential for cross-transmission of diseases between cultured and wild fish populations in Inner Ambon Bay.

KEYWORDS: Inner Ambon Bay; *Lates calcarifer*; *parasite*; *Vibrio* spp.

ABSTRAK: Dinamika dan Risiko Transmisi Silang Penyakit pada Ikan Kakap Putih (*Lates calcarifer*) Budidaya dan Liar di Teluk Ambon Dalam, Indonesia

Penelitian ini bertujuan mengevaluasi potensi infeksi bakteri ***Vibrio*** *spp. dan parasit pada ikan kakap putih (****Lates calcarifer****) budidaya dan ikan liar di perairan Teluk Ambon Dalam. Penelitian dilaksanakan selama lima bulan (Agustus–Desember 2024) dengan metode purposive sampling, masing-masing lima ekor ikan kakap putih budidaya dan lima ekor ikan liar setiap bulan. Isolasi bakteri dilakukan dari organ hati dan ginjal menggunakan media SWC agar dan TCBS agar, sedangkan identifikasi bakteri menggunakan kit API 20 NE. Identifikasi parasit dilakukan melalui pemeriksaan mikroskopis pada organ target, yaitu insang, kulit, dan usus. Hasil penelitian menunjukkan bahwa* ***Vibrio*** *spp. ditemukan dengan kelimpahan relatif tinggi, didominasi oleh* ***V. harveyi****,* ***V. alginolyticus****, dan* ***V. vulnificus****. Jenis Parasit yang teridentifikasi meliputi* ***Trichodina*** *sp.,* ***Cryptocaryon irritans****,* ***Benedenia*** *sp.,*

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dan nematoda. Prevalensi infeksi parasit pada ikan budidaya berkisar 40–70%, sedangkan pada ikan liar mencapai 60%. Hasil penelitian ini mengindikasikan adanya potensi penularan silang penyakit antara ikan budidaya dan ikan liar di Teluk Ambon Dalam.

KATA KUNCI: Teluk Ambon Dalam; kakap putih; parasit; *Vibrio* ***spp.***

INTRODUCTION

Barramundi (*Lates calcarifer*), also known as Asian sea bass, is a major aquaculture commodity in the Asia–Pacific region, including Indonesia. This species has high economic value, rapid growth, and broad tolerance to environmental conditions, making it widely cultivated in marine floating net cage systems in coastal waters and bays (FAO, 2022). Inner Ambon Bay is a strategic location for aquaculture development because of its relatively calm waters and favorable environmental conditions (Abukasim *et al*., 2021).

However, the intensification of aquaculture in open systems increases vulnerability to infectious diseases. Diseases are a major limiting factor in mariculture and can cause significant economic losses (Borut *et al.,* 2024; Lafferty *et al*., 2015; Samsing *et al*., 2023). Among bacterial pathogens, the genus *Vibrio* represents opportunistic bacteria commonly associated with vibriosis in barramundi. Several species, such as *V. alginolyticus*, *V. harveyi*, *V. parahaemolyticus*, and *V. vulnificus*, are known to cause systemic infections and internal organ damage (Austin & Zhang, 2006). The incidence of vibriosis generally increases under conditions of high temperature, poor water quality, and high stocking density, all of which are common in intensive farming (Cowan *et al*., 2024).

External parasites also represent a serious threat. Species such as *Trichodina* sp., *Cryptocaryon irritans*, and *Neobenedenia* sp. have been reported to cause irritation, epithelial tissue damage, chronic stress, and increased susceptibility to secondary infections (Cervera *et al.,* 2022; Reyes-Mero *et al.*, 2024; Liang *et al.*, 2025. Parasite prevalence is relatively high in tropical and subtropical waters, particularly in aquaculture systems with high stocking density.

Another important issue is the potential cross-transmission of diseases between cultured and wild fish. Wild fish surrounding aquaculture cages may act as natural reservoirs for various pathogens, maintaining and spreading infectious agents in the aquatic environment. Unlike cultured fish, which are usually monitored and managed under controlled conditions, wild fish are continuously exposed to diverse environmental stressors and microbial communities, increasing their likelihood of harboring opportunistic pathogens. These pathogens, such as *Vibrio* spp. or ectoparasites, can persist in wild populations and later infect cultured fish through shared water, feed, or fecal contamination (Bouwmeester *et al*., 2021; Harrington *et al*., 2023). The risk of this cross-infection process is exacerbated when biosecurity measures, such as water filtration, disinfection, and cage distance management, are not effectively implemented. Therefore, understanding the role of wild fish as pathogen reservoirs is crucial for developing integrated disease management strategies and ensuring the sustainability of aquaculture systems (Manchanayake *et al.*, 2023)

To date, studies on the disease dynamics of *L. calcarifer*, including both cultured and wild fish populations, are still limited, particularly in Inner Ambon Bay and, more generally, in Indonesia. Therefore, this study aimed to evaluate the presence and prevalence of *Vibrio* spp. and parasite infections in cultured and wild barramundi in the region. The results are expected to serve as a scientific baseline for developing more effective disease-prevention and control strategies in mariculture systems.

MATERIALS AND METHODS

Study Period and Location

The research was conducted in Inner Ambon Bay, Indonesia, a major center for marine fish farming using floating net cages (FNC). Sample analyses were performed at the Aquaculture Laboratory, Faculty of Fisheries and Marine Science, Universitas Pattimura Ambon, and at the Fish Health and Disease Laboratory, Marine Aquaculture Development Center Waiheru, Ambon. The study lasted five months, from August to December 2024. Sampling sites for fish collection and in situ water quality measurement are shown in Figure 1 (Located at the sites: Red and white brigde, Marta Afons, Galala, Halong, and Waiheru, which are marked with yellow dots).

Research Design and Sampling Technique

A purposive sampling method was applied to obtain representative barramundi (*Lates calcarifer*) from two populations: cultured fish maintained in floating net cages and selected based on their life-cycle stage and abnormal physical condoction, whereas wild fish were manually captured around the cages using handlines with bait. Each month during the five-month observation period, five cultured fish and five wild fish were sampled according to the fish life cycle, resulting in a total of 50 fish specimens.

Water Quality Measurement

Water quality parameters were measured *in situ* at the floating net cage sites and at wild fish capture locations (wild fish caught around the cages, excluding the cultured fish). The measured parameters included temperature (°C), salinity (ppt), pH, dissolved oxygen (DO, mgL^{-1}), and ammonia (NH_3-N, mgL^{-1}). These measurements were obtained using a YSI ProDSS Multiparameter Water Quality Meter (USA) to record temperature, salinity, pH, and DO, while ammonia concentrations were determined using an API Ammonia Test Kit (USA).

Clinical and Morphological Observation

Captured fish were examined visually to identify clinical signs of disease, including changes in body color, skin lesions or ulcers, abdominal swelling, and abnormal behavior (e.g., slow swimming or surface aggregation). Morphological parameters, such as total length and body weight, were systematically recorded to support the assessment of fish health and physical condition.

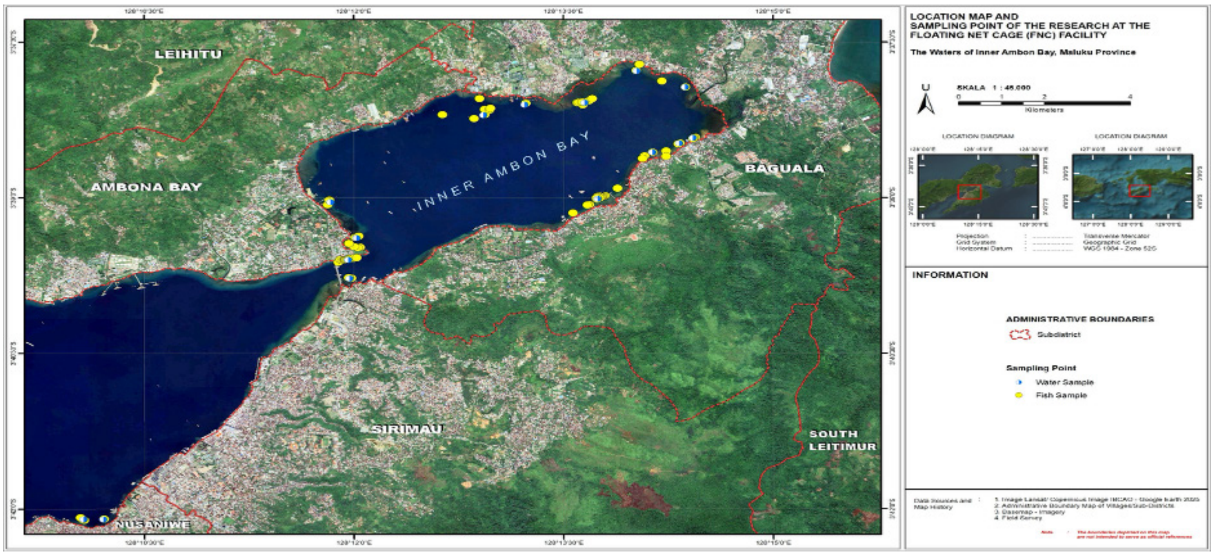

Figure 1. Sampling sites for fish collection and in situ water quality measurement.

Bacterial Isolation and Identification

Liver and kidney tissues were sampled. Organ surfaces were sterilized, and tissues were aseptically excised. Samples were inoculated on sea water complete (SWC) agar and tiosulfate citrate bile-salt sucrose (TCBS) agar, and incubated at 28–30°C for 24–48 h. Colonies were identified using the Analytical Profile Index (API) 20 Non-Enteric (NE) system (bioMérieux, France) to determine *Vibrio* species based on biochemical profiles (Austin & Austin, 2016).

Parasitological Examination

Gill, skin, and intestinal tissues were examined for parasites. Wet mounts were prepared in physiological saline and observed under a light microscope at appropriate (40–400x). Parasites were identified morphologically with reference to tropical fish parasite identification keys (Woo, 2006)

Data Analysis

The total bacterial count and total *Vibrio* count were calculated using the spread plate method with the formula:

$$\sum bacteria \;=\; \frac{N}{(\sum spread)} \; x \; \frac{1}{F} \text{.....................................(1)}$$

where:
∑ bacteria = number of bacterial cells (CFU/g)
N = number of colonies
F = dilution factor

Total plate count (TPC) for bacteria and *Vibrio* spp. was analyzed quantitatively. Data were subjected to analysis of variance (ANOVA) at a 95% confidence level using SPSS version 25.0 (IBM Corp., USA). When significant differences ($p < 0.05$) were detected, Tukey's HSD test was applied to determine significantly different groups. Identification of *Vibrio* species from biochemical and API 20 NE kit results was analyzed descriptively.

For parasites, prevalence and intensity of infection were calculated using equations (1) and (2):

$$Prevalence\ (\%) \;=\; \frac{Number\ of\ infected\ fish}{Number\ of\ examined\ fish}\ \text{x100} \text{ ....(2)}$$

$$Intensity \;=\; \frac{Total\ number\ of\ parasites\ in\ infected\ fish}{Number\ of\ infected\ fish} \text{.......(3)}$$

Accordingly, the results were presented in descriptive tables and graphs to illustrate the infection levels in cultured and wild fish throughout the study period.

RESULTS AND DISCUSSION

Water Quality

Observations of water quality parameters in the Inner Ambon Bay during the research period from August to December 2024 revealed significant dynamics in the physicochemical characteristics of the aquatic environment. As shown in Table 1, most parameters, such as temperature, salinity, and pH, remained within the recommended threshold range for tropical marine waters. However, two critical parameters, namely dissolved oxygen (DO) and ammonia (NH_3), exhibited significantly abnormal values, indicating a progressive decline in environmental water quality.

Water temperature is a critical abiotic factor that regulates nearly all biological, chemical, and ecological processes in aquaculture systems. The observed gradual increase in temperature from 28.5°C (August) to 30.5°C (December) indicates seasonal warming, likely driven by increased solar irradiance and reduced precipitation during the dry season. Although this temperature range falls within the optimal thermal window (26–32°C) for tropical marine species, values exceeding 30°C approach the upper physiological threshold and may trigger thermal stress (Table 1). In addition, elevated water temperature decreases oxygen solubility while simultaneously increasing the metabolic oxygen demand in fish. Nilsson *et al.* (2009) found that in coral reef fishes, the aerobic scope, the capacity for aerobic performance, was reduced by nearly 50% when water temperature increased from 29°C to 33°C, particularly in thermally sensitive species. This decline directly disrupts key physiological functions, including growth, reproduction, and immune responses.

Salinity increased from 32.0 ppt to 34.0 ppt over the study period, likely due to increased evaporation and reduced freshwater inflow during the dry season. While marine fish species generally tolerate salinity within this range, rapid or concurrent fluctuations in salinity alongside other environmental stressors can lead to secondary osmotic stress. Ngarari *et al.* (2024) reported that both salinity and temperature significantly affect the oxygen consumption of *Artemia franciscana*, with temperature being the more dominant factor. The study highlighted the synergistic effects of salinity and temperature on metabolic performance, which is highly relevant for aquaculture operations in fluctuating environments.

In open systems, proactive salinity management strategies, including controlled water exchange, pond shading, or constructed wetlands, may help stabilize the aquatic environment, especially during seasonal transitions. The recorded pH range of 7.6 to 8.2 indicates a neutral to slightly alkaline environment, ideal for supporting photosynthesis and the proliferation of phytoplankton and beneficial microbes. However, in aquaculture systems, pH interacts with other parameters, particularly ammonia, in complex and potentially harmful ways. At higher pH (>8.0) and temperature, the proportion of unionized ammonia (NH_3), the toxic form, increases significantly. Therefore, pH indirectly influences ammonia toxicity and must be carefully monitored alongside total ammonia nitrogen (TAN) concentrations. Elevated NH_3 levels can disrupt gill function, impair ion regulation, and suppress immune responses in cultured fish species. From an aquaculture management perspective, buffering agents and liming schedules must be carefully calibrated to prevent pH spikes, particularly during phytoplankton blooms or during intense daytime photosynthetic activity.

A sharp decline in dissolved oxygen (DO) from 5.2 mg L^{-1} in August to 3.0 mg L^{-1} in December indicates a progression toward hypoxic conditions, particularly during the dry season. DO levels below 5.0 mg L^{-1} are considered suboptimal for most marine organisms, while levels under 3.0 mg L^{-1} are indicative of oxygen stress and can be fatal to sensitive species. Several factors likely contributed to the DO reduction: higher

Table 1. Mean water quality parameters in Inner Ambon Bay during the study period (August-December 2024)

Parameters	Month					Normal Range	References
	Aug	Sept	Oct	Nov	Dec		
Temperature (°C)	28.5	29.0	29.8	30.2	30.5	26–32	Boyd & Tucker (2012)
Salinity (ppt)	32.0	32.5	33.0	33.5	34.0	28–34	Effendi (2003)
pH	7.6	7.8	7.9	8.0	8.2	7.5–8.5	Effendi (2003)
Dissolved Oxygen (mg L^{-1})	5.2	4.8	4.0	3.5	3.0	> 5	Boyd & Tucker (2012)
Ammonia (mg L^{-1})	0.12	0.15	0.20	0.26	0.32	< 0.1	Effendi (2003); Boyd & Tucker (2012)

temperatures reduce oxygen solubility; aerobic decomposition of organic matter increases microbial oxygen consumption; and reduced vertical mixing or stratification limits reoxygenation in deeper layers.

Pörtner and Knust (2007) formulated the concept of Oxygen- and Capacity-Limited Thermal Tolerance (OCLTT), which explains how oxygen availability and circulatory efficiency define the thermal tolerance limits of marine ectotherms. In relation to the findings of this study, a decrease in dissolved oxygen (DO) was observed in warm aquaculture environments (28–30°C) with high organic matter content, where intensified microbial respiration suppressed aerobic fish metabolism and weakened their immune systems. Under such conditions, the *Vibrio* population tends to increase, elevated temperatures accelerate bacterial growth, organic residues from feed serve as nutrient sources, and low DO levels promote microbial respiration, which is typically associated with higher *Vibrio* abundance. Based on the obtained data, a correlation was identified between DO (mg L^{-1}) and *Vibrio* abundance (CFU mL^{-1}) across sites and sampling periods, showing a generally negative correlation (as DO decreases, *Vibrio* abundance increases).

To mitigate DO stress, aquaculture systems should integrate aeration technologies, optimize stocking densities, and employ real-time monitoring systems for adaptive management. Ammonia levels increased from 0.12 mg L^{-1} to 0.32 mg L^{-1}, exceeding the generally accepted safe threshold of < 0.1 mg L^{-1}. This indicates organic pollution, most likely derived from uneaten feed, fish waste, and potential domestic runoff in the surrounding area. In aquaculture, ammonia toxicity is a primary constraint on water quality and fish health.

Parvathy *et al.* (2023) found that tropical fish exposed to high ammonia concentrations at elevated temperatures experienced suppressed metabolic rates and heightened sensitivity to oxygen stress. Their findings confirm that ammonia toxicity is exacerbated by warming and hypoxia, a situation highly relevant to tropical aquaculture. Increased ammonia also contributes to eutrophication, triggering harmful algal blooms and further depleting oxygen levels. Effective strategies to control ammonia include the use of nitrifying probiotics, biofilters, and reducing feed waste through precision feeding protocols.

The seasonal changes observed in water temperature, dissolved oxygen, and ammonia concentrations collectively pose a serious threat to the sustainability of tropical marine aquaculture systems. Without adequate intervention, these shifts can disrupt ecosystem functions and lead to production losses.

Clinical Signs

Observations of clinical symptoms in barramundi (*L. calcarifer*) revealed a significant difference between farmed and wild fish populations (Table 2). Farmed fish exhibited a higher frequency of clinical manifestations, reflecting greater environmental stress and a higher potential for infection within the open aquaculture systems of Inner Ambon Bay.

The appearance of red lesions or ulcers on the skin and fins, recorded only in farmed fish, is strongly associated with secondary bacterial infections, most notably *Vibrio* spp. These pathogens are opportunistic and tend to proliferate when fish are exposed to environmental stressors, such as hypoxia, elevated ammonia levels, or handling injuries. According to Manchanayake *et al.* (2023), lesions in farmed marine fish often develop due to epithelial breakdown triggered by crowding, mechanical abrasions, and immunosuppression, creating entry points for bacteria.

Pallor of the gills, observed in both farmed and wild populations but more frequently in farmed *L. calcarifer*, strongly suggests underlying physiological disturbances such as anemia, gill tissue damage, or chronic respiratory distress. This condition may result from systemic disorders that reduce erythrocyte count or impair oxygen transport, as well as from local tissue damage caused by environmental stressors or pathogens. Anemia in fish is often characterized by pale gills and can be induced by bacterial infections, blood-sucking parasites, or exposure to toxic compounds (PetMD, 2023; Roberts, 2012). Poor water quality, particularly low dissolved oxygen (DO) and elevated ammonia, has been shown to exacerbate gill damage; prolonged hypoxia can lead to lamellar fusion and epithelial degeneration, impairing gas exchange and producing visibly pale or congested gills (Currie *et al*., 2014; FAO, 2021). In addition, ectoparasitic infestations, such as *Neobenedenia* spp., have been documented to cause gill inflammation, discoloration, and respiratory impairment, especially in cultured marine fish species (Hirazawa *et al*., 2016; Whittington, 2004). The higher frequency of gill pallor observed in cultured barramundi may therefore be attributed to multiple stressors, including high stocking density, limited water exchange, and increased exposure to pathogens and organic load. Understanding these interactions is essential for improving biosecurity and disease management practices in aquaculture systems.

These findings align with Vo *et al.* (2020), who identified pale gills in barramundi (*L. calcarifer*) as a clinical biomarker of oxygen deficiency and haematological imbalance in poorly aerated cage systems. Their study demonstrated that suboptimal aeration leads to reduced dissolved oxygen levels, which, in turn, disrupt erythropoiesis and lower haemoglobin concentration, resulting in visible gill pallor and lethargic behaviour. Prolonged hypoxic conditions were also associated with increased stress indicators, such as elevated cortisol levels and reduced immune cell counts, indicating that oxygen limitation not only affects respiration but also compromises the overall physiological resilience of fish. In aquaculture environments with high stocking density and limited water circulation, such conditions can easily develop, amplifying the risk of secondary infections and mortality. Therefore, the observation of pale gills in the present study reinforces the importance of maintaining adequate aeration and oxygenation in cage-culture systems to ensure optimal fish health and productivity.

Swollen abdomens, observed only in farmed fish, may indicate systemic infections, enteritis, or ascites. These signs often stem from bacterial septicemia or parasitic infestation in the gastrointestinal tract or liver. In intensive aquaculture, infections by *Streptococcus iniae*, *Photobacterium damselae*, or *Myxosporea* species have been reported as common causes of abdominal swelling. Yue and Guo (2025) observed similar pathology in barramundi reared in open cages, reporting liver necrosis, hemorrhagic intestines, and bacterial isolation from peritoneal fluid.

Mucus hypersecretion, prominent in farmed fish, reflects the host’s response to irritation, ectoparasite attachment, or poor water quality (e.g., high ammonia or suspended solids). The mucus layer plays a

Table 2. Comparison of clinical signs between cultured (*Lates calcarifer*) and wild fish

Clinical Symptoms	Description	Farmed	Wild Fish
Lesion/Ulcer	Red lesions or ulcers on the skin and fins	+	-
Pale gills	Gill discoloration, indicative of anemia/stress	+	+
Swollen abdomen	Indication of systemic infection or fluid accumulation	±	-
Excess mucus production	Thick mucus layer on the body surface, response to irritation	+	±
Slow movement	Fish appear weak, swim passively or near the surface	+	±
Skin hemorrhage	Red spots on the skin due to local bleeding	±	-
Damaged/eroded fins	Fin edges torn or eroded	±	-

Note: (+) frequently observed; (±) occasionally observed; (–) not observed.

central role in non-specific immunity, as it contains antimicrobial peptides, lysozymes, and immunoglobulins. Shinn *et al.* (2015) showed that farmed barramundi exposed to *Trichodina* spp. and *Benedenia* spp. displayed thicker mucus and epidermal hyperplasia, highlighting its diagnostic significance in parasite-prone environments.

Sluggish movement or surface-dwelling behaviors, predominantly seen in farmed fish, indicate hypoxia, neurological compromise, or severe systemic infection. Behavioral alterations are among the earliest observable signs of stress or illness in aquaculture settings. MacAulay *et al.* (2022) emphasized the importance of behavioral indicators in early disease detection protocols. Fish exposed to sublethal levels of ammonia or low DO commonly exhibit reduced swimming activity, decreased feed intake, and surface gasping.

Although less frequent, skin hemorrhages and fin erosion occurred more often in farmed fish. These signs often result from mechanical trauma, due to crowding or aggressive interactions; parasitic erosion, especially from monogeneans and copepods; bacterial hemorrhagic septicemia, caused by *Aeromonas* spp. or *Edwardsiella tarda*. Yue and Guo (2025) linked such symptoms to poor cage design and parasitic infestations in high-density barramundi farming, advocating for integrated health management and regular parasitological screening.

Figure 2 presents a visual comparison of the clinical conditions of barramundi (*L. calcarifer*), ranging from healthy individuals to those suffering from parasitic and bacterial infections. These external morphological signs serve as practical non-invasive diagnostic indicators of physiological health, environmental stress, and pathogen presence in aquaculture environments, particularly in open systems exposed to fluctuating water quality and high biological loads.

Figure 2(A) shows a healthy barramundi, characterized by: bright, even body coloration, intact, non-eroded fins, no visible skin lesions, ulceration, or signs of irritation. Such features strongly indicate a stable environment, free from major stressors such as low oxygen, ammonia, or pathogen load. It also suggests an active and functioning immune system. According to MacAulay *et al.* (2022), these visual indicators reflect fish living under optimal water quality conditions, where stress is minimal, and immune homeostasis is maintained.

Figure 2(B) depicts a fish showing classic signs of ectoparasite infection, such as: *Cryptocaryon* sp. (marine white spot), *Trichodina* sp. (protozoa affecting gill/skin epithelium), *Benedenia* sp. (monogenean fluke attacking skin and fins). Clinical symptoms observed include: skin hemorrhages, often

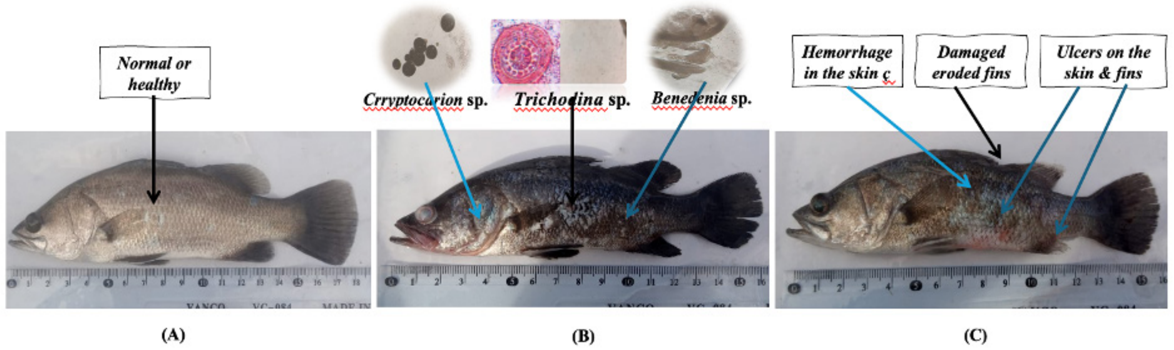

Figure 2. Clinical conditions of barramundi (*L. calcarifer*): (A) Normal, healthy fish showing bright body coloration and intact fins without lesions; (B) Fish exposed to parasites (*Cryptocaryon* sp., *Trichodina* sp., and *Benedenia* sp.) exhibiting skin hemorrhage and external irritation; (C) Fish exposed to *Vibrio* spp. showing hemorrhages on the skin, eroded fins, and ulcerative lesions consistent with vibriosis symptoms

from localized parasite attachment; surface irritation leading to erratic swimming; and excessive mucus production, a common innate immune response to ectoparasitic irritation.

These infestations are usually reported in systems with degraded water quality, including high ammonia levels, overcrowding, and poor hygiene practices. Buchmann (2022) found that *Trichodina* and *Benedenia* infestations in farmed barramundi led to epidermal hyperplasia, increased mucus production, and reduced growth performance. Similarly, Sufardin *et al.* (2021) reported *Benedenia* sp. as a major contributor to fin erosion and stress-related immunosuppression.

Figure 2(C) shows a fish displaying severe clinical symptoms of vibriosis, including: extensive skin hemorrhaging, eroded fins and exposed tissue, open skin ulcers, often secondary to prior parasitic damage. Vibriosis, caused by *Vibrio* spp. (e.g., *V. harveyi*, *V. alginolyticus*), is a common bacterial disease in tropical aquaculture and is usually opportunistic. It is most frequently triggered when fish are stressed by suboptimal environmental conditions, such as elevated water temperatures, low dissolved oxygen (DO), and concurrent parasitic infestations.

Kim and Chun (2021) highlighted that outbreaks of vibriosis are often linked to seasonal DO drops and rising temperatures, which reduce fish immune competence. Emphasized that skin damage from parasites or handling increases susceptibility to *Vibrio* infections, which can lead to high mortality if left untreated.

A visual comparison across panels clearly demonstrates that fish exposed to parasites and bacterial pathogens in aquaculture settings exhibit significantly more severe external tissue damage than healthy individuals. These observations are consistent with the clinical symptom data presented in Table 2, which show that farmed fish have higher incidences of lesions, hemorrhage, and fin erosion than wild fish.

Microbiological identification revealed the presence of three *Vibrio* species such as *V. harveyi*, *V. alginolyticus*, and *V. vulnificus*, in both cultured and wild *L. calcarifer* populations, with varying prevalence rates (Table 3).

Vibrio harveyi was identified as the most prevalent species, occurring in 60% of farmed fish and 45% of wild fish. This species is recognized as a primary causative agent of vibriosis in marine finfish aquaculture, especially in tropical, high-density farming systems. Manchanayake *et al.* (2023) confirmed its role as a dominant pathogen, frequently associated with outbreaks in barramundi and groupers raised under intensive conditions. The clinical symptoms observed in this study, skin ulcers, hemorrhaging, and fin erosion, are consistent with the typical manifestations of *V. harveyi* infection. These match the external symptoms documented earlier in Table 2 and Figure 2, including fin damage and systemic stress, which are exacerbated by low dissolved oxygen (DO) and poor water quality.

Vibrio alginolyticus opportunistic but environmentally driven detected in 50% of farmed fish, and 30% of wild fish, *V. alginolyticus* is generally a commensal marine bacterium, but becomes opportunistically pathogenic in suboptimal environmental conditions, especially when fish are injured or stressed. Sanches-Fernandes *et al.* (2022) emphasized that elevated ammonia levels, increased turbidity, and excessive handling during

Table 3. Prevalence of *Vibrio* spp. bacteria in farmed barramundi (*L. calcarifer*) and wild fish in the Inner Ambon Bay

No	Bacterial Species	Farmed Barramundi (%)	Wild Fish (%)
1	*Vibrio harveyi*	60	45
2	*Vibrio alginolyticus*	50	30
3	*Vibrio vulnificus*	40	25

netting or feeding contribute to the transition of *V. alginolyticus* from benign to pathogenic status. Its higher detection rate in cultured fish suggests that the increased organic loading, uneaten feed, and fecal accumulation in aquaculture environments create favorable conditions for its proliferation.

Vibrio vulnificus: less common, but highly virulent. Though less frequently isolated, *V. vulnificus* was found in 40% of farmed fish and 25% of wild fish. This species is known for its high virulence and zoonotic potential, capable of causing serious infections in both fish and humans. Its prevalence is closely associated with elevated water temperatures and hypoxic conditions, which are common during the late dry season in tropical coastal waters. Hernández-Cabanyero *et al.* (2020) demonstrated that *V. vulnificus* growth and pathogenicity increase sharply at temperatures above 30°C.

Microscopic examination of the gills, skin, fins, and gastrointestinal tract of *L. calcarifer* populations from Inner Ambon Bay revealed infection by four major parasitic taxa. These were *Trichodina sp.*, *C. irritans*, *Benedenia sp.*, and an unidentified intestinal nematode. The infection site specificity and prevalence rates, as presented in Table 4 and Figure 3, offer essential insights into environmental risk factors associated with open aquaculture systems.

Trichodina sp. protozoan ectoparasite with highest prevalence in farmed fish: 50%, wild fish: 40% and infection site: gills and skin. *Trichodina* spp. are ciliate protozoa frequently found in aquaculture systems, especially under poor water quality and high organic load. They attach to epithelial tissues, causing mucosal irritation, epithelial damage, and respiratory distress. Their high prevalence in farmed fish reflects environmental stressors such as

Table 4. Prevalence of parasites in farmed barramundi (*L. calcarifer*) and wild fish in the Inner Ambon Bay

No	Parasite Species	Infection Site	Farmed Barramundi (%)	Wild Fish (%)
1	*Trichodina* sp.	Gills, skin	50	40
2	*C. irritans*	Gills	40	35
3	*Benedenia* sp.	Skin, fins	30	25
4	Nematoda (unspecified)	Intestine	20	30

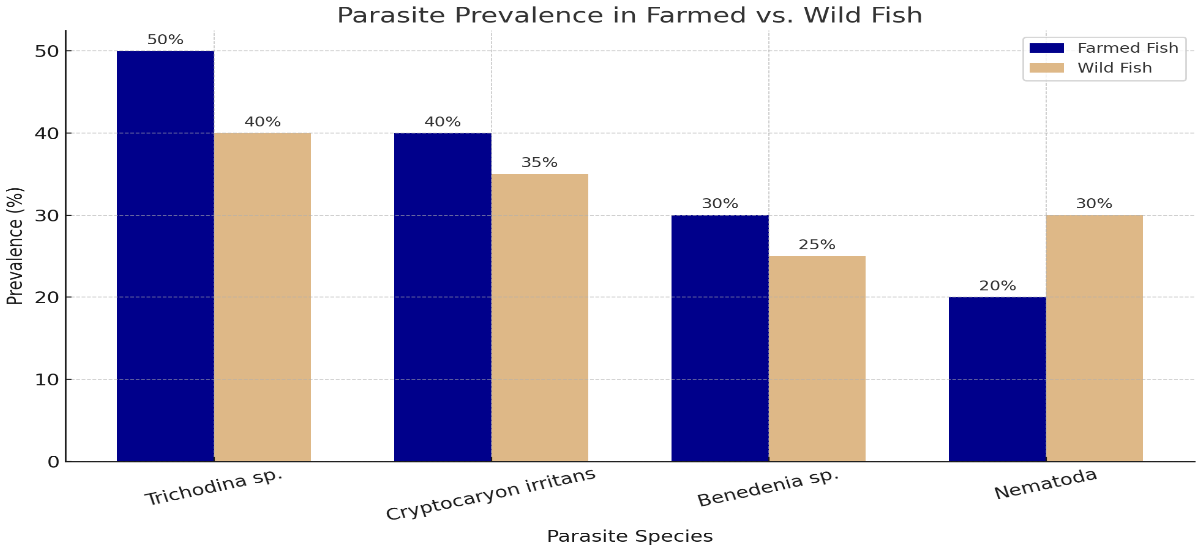

Figure 3. Prevalence (%) of parasites in cultured (*L. calcarifer*) and wild in Inner Ambon Bay

elevated ammonia and reduced dissolved oxygen in net cages. Qian *et al.* (2022) confirmed the strong correlation between *Trichodina* infection and mucus hypersecretion and reduced feed efficiency in intensively cultured fish.

Cryptocaryon irritans – causative agent of marine white spot disease farmed fish: 40%, wild fish: 35% and infection site: gills. *Cryptocaryon irritans* is a marine protozoan responsible for "white spot disease" particularly in juvenile or immunocompromised fish. This parasite causes epithelial cell lysis, compromising gill function and reducing oxygen uptake. Yin *et al.* (2014) reported that infection is often triggered by stocking density, temperature spikes, and handling stress, all common in aquaculture operations. Its slightly higher presence in farmed populations supports this association.

Benedenia spp., are monogenean flatworms that can cause ulcerative skin lesions and fin erosion in fish, which could serve as entry point wounds for bacterial pathogens such as *Vibrio spp*. In this study, the parasite infested 30% and 25% in farmed and wild fish. Brazenor and Hutson (2015) demonstrated that *Benedenia* thrives in calm, nutrient-rich environments, such as floating cages with low water exchange rates, enabling rapid reproduction and reinfection cycles.

Nematodes (unspecified) are intestinal endoparasites found in both wild and farmed fish. The prevalence of nematode infection is recorded at 20% in farmed fish and 30% in wild fish, with the primary site of infection being the gastrointestinal tract. Nematodes are the only parasite group that shows a higher prevalence in wild fish than in farmed fish. As endoparasites, nematodes rely on natural food chains, such as small fish, crustaceans, or zooplankton, which act as intermediate hosts. These organisms are generally absent from artificial feeds formulated for aquaculture systems, so the chance of infection in farmed fish is relatively low. Pereira and González-Solís (2022) found nematodes common in wild-caught marine fish in tropical ecosystems, especially those feeding on a diverse natural diet.

Figure 3 shows that *Trichodina* sp., *C. irritans*, and *Benedenia* sp. are more frequently found in cultured fish, whereas nematodes are more dominant in wild fish. This pattern reflects the environmental and ecological differences between aquaculture systems and natural habitats. Open aquaculture systems tend to promote higher ectoparasite prevalence. The prevalence of ectoparasites can be influenced by several factors, including high stocking density, nutrient accumulation (such as uneaten feed and waste), and frequent fish handling. In contrast, natural environments are more conducive to the life cycles of endoparasites, which are influenced by trophic complexity and predator–prey interactions.

This parasitic burden correlates strongly with clinical observations in Table 2, showing excess mucus, lesions, and abnormal swimming, and in Figure 2, which displays visual signs of irritation, tissue erosion, and ulceration in farmed fish. These clinical manifestations underscore the impact of ectoparasitic infestation on the external morphology and welfare of cultured fish.

The analysis of infection intensity, measured as the mean number of parasites per infected individual, revealed notable differences between farmed and wild *L. calcarifer* in Inner Ambon Bay. According to Table 5 and Figure 4, farmed fish exhibited significantly higher infection intensities for nearly all types of ectoparasites, while wild fish showed higher intensity only for nematode infections.

Trichodina sp., exhibited the highest infection intensity, with an average of 15 parasites per individual in farmed fish and 10 in wild fish. This finding highlights that high stocking densities and poor water quality in aquaculture environments greatly facilitate the proliferation of this ectoparasite. *Trichodina* is widely recognized as a bioindicator of poor water sanitation and thrives in water columns with elevated organic matter content (Al-Marjan & Abdullah, 2015).

Cryptocaryon irritans, the causative agent of marine white spot disease, showed an infection intensity of 12 parasites per individual in farmed fish and nine in wild fish. This protozoan parasite can reproduce rapidly in enclosed environments with limited water circulation, such as floating net cages. According to Guo *et al.* (2023), high intensities of *C. irritans* lead to severe damage of gill epithelia and impaired respiration, particularly in fish already stressed by environmental factors.

Benedenia sp., a monogenean ectoparasite commonly found in marine aquaculture systems, exhibited an infection intensity of nine parasites per individual in farmed *L. calcarifer* and seven in wild individuals. This parasite has a direct life cycle, enabling rapid transmission under the high host densities typical of aquaculture environments. According to Wijaya *et al*. (2019), *Benedenia* thrives in systems with limited water exchange and frequent host contact, such as floating net cages, where environmental stress and high organic loads facilitate parasite proliferation. The parasite attaches firmly to the skin and fins using specialized haptoral hooks, which can cause mechanical injuries that compromise the epithelial barrier. These open wounds not only disrupt osmoregulation but also serve as entry points for opportunistic bacteria, increasing the likelihood of secondary infections by pathogens such as *Vibrio* spp. and *Aeromonas* spp., thereby elevating mortality risk. Consequently, effective monitoring and management of *Benedenia* infestations are crucial to maintaining fish health and preventing epizootic outbreaks in intensive aquaculture systems.

The only exception to the general trend

Table 5. Intensity of parasitic infections in farmed barramundi (*L. calcarifer*) and wild fish in the Inner Ambon Bay

No	Parasite Species	Infection Site	Intensity in Farmed Fish (mean parasites/individual)	Intensity in Wild Fish (mean parasites/individual)
1	*Trichodina* sp.	Gills, skin	15	10
2	*C. irritans*	Gills	12	9
3	*Benedenia* sp.	Skin, fins	9	7
4	Nematoda (unspecified)	Intestine	6	8

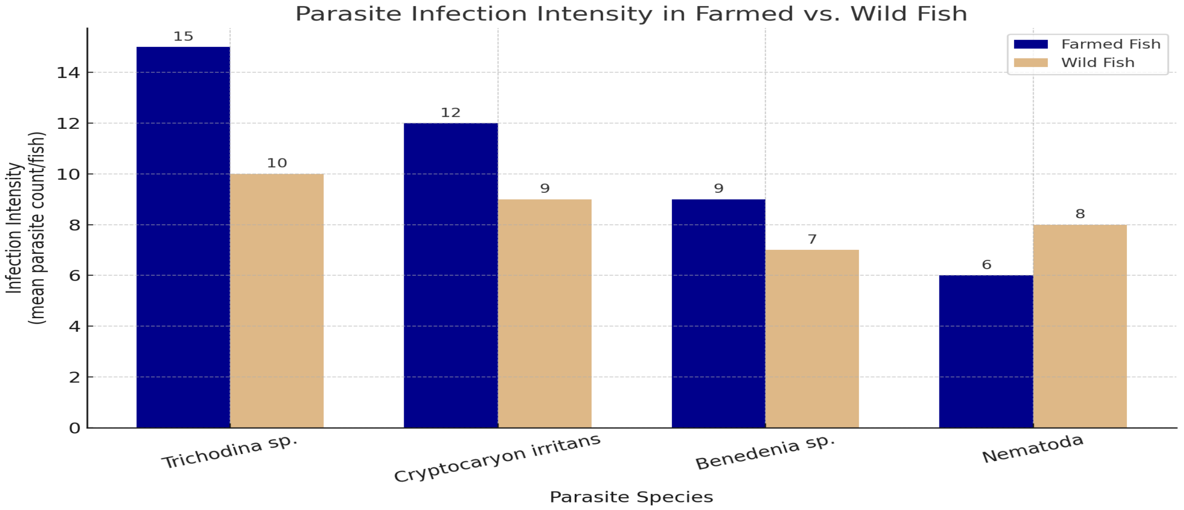

Figure 4. Mean intensity of different parasitic infections in cultured (*L. calcarifer*) and wild in Inner Ambon Bay

was observed in nematode infections, where wild fish exhibited a higher intensity (eight parasites per individual) compared to farmed fish (six parasites per individual). This discrepancy may be attributed to the natural diet of wild fish, which includes benthic organisms and invertebrates that often serve as intermediate hosts for nematodes. As noted by Menconi *et al.* (2021), the broader feeding spectrum in wild fish increases the likelihood of completing the nematode life cycle.

Figure 4 clearly shows these differences, indicating that ectoparasites (*Trichodina*, *Cryptocaryon*, *Benedenia*) are more intense in farmed fish, whereas nematodes are more prevalent in wild fish. These results confirm that intensive aquaculture systems elevate the risk of ectoparasite accumulation, whereas natural waters pose a greater threat from endoparasitic infections. This pattern is consistent with the clinical symptoms observed in Table 2 and Figure 2, which indicate more severe external tissue damage in farmed fish. Therefore, implementing effective management strategies, such as controlling stocking density, improving water circulation, and regularly cleaning substrates, is essential to minimize parasitic outbreaks in marine aquaculture systems.

The monthly monitoring of Vibrio spp. abundance in barramundi from both aquaculture systems and wild habitats in Inner Ambon Bay revealed a consistent upward trend in bacterial concentrations between August and December 2024. Farmed fish consistently exhibited higher bacterial loads compared to their wild counterparts, with the most significant differences observed during the late dry season (November–December) (Figure 5).

Dominance of *V. harveyi* in floating net cage aquaculture systems in Inner Ambon Bay. In farmed fish, *V. harveyi* reached a peak abundance of 6.0 log CFU g^{-1} in December, which was significantly higher than that of *V. alginolyticus* (5.3 log CFU g^{-1}) and *V. vulnificus* (4.8 log CFU g^{-1}). ANOVA and Tukey's post hoc tests indicated that the differences among these bacterial species were statistically significant ($p < 0.05$). These findings are consistent with those of Zhang *et al.* (2020), who identified *V. harveyi* as the primary causative agent of vibriosis in tropical marine aquaculture systems, particularly under conditions of high stocking density and poor water quality.

Environmental conditions supporting *Vibrio* proliferation, the increase in *Vibrio* spp. abundance is associated with declines in dissolved oxygen (DO), increases in ammonia concentration, and higher water temperatures (Table 1). These environmental shifts are

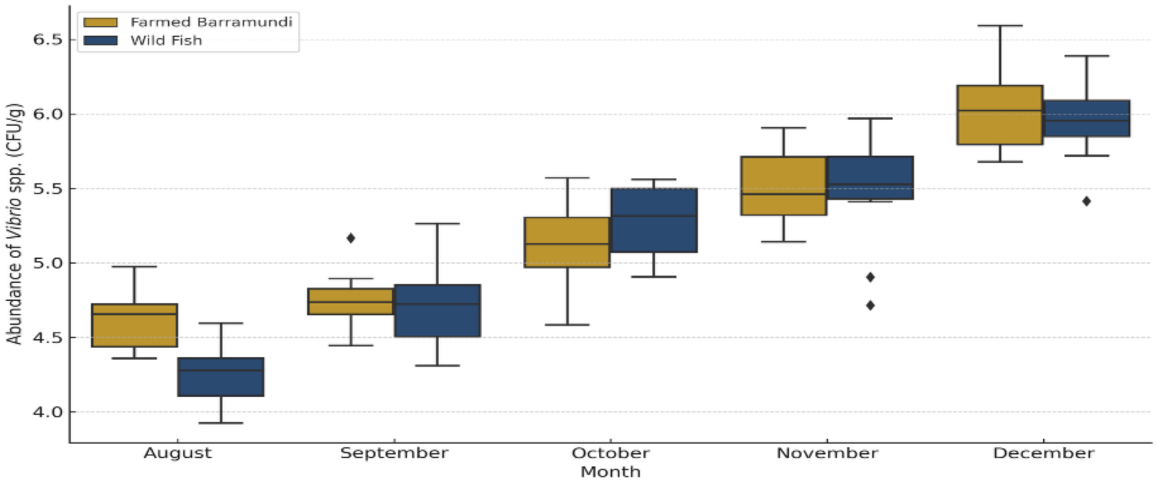

Figure 5. Distribution of *Vibrio* spp. abundance in cultured and wild barramundi (*L. calcarifer*) from August to December 2024

known to trigger stress responses in fish, compromising immune function and creating a favorable niche for opportunistic pathogens (Zhang *et al.,* 2022). Specifically, low DO and high organic load promote bacterial respiration and biofilm formation, conditions under which *V. harveyi* thrives.

Clinical signs such as ulceration, haemorrhage, and fin erosion in barramundi (*L. calcarifer*), caused by interaction of parasitic and bacterial agents (Table 2, Figure 2) are consistent with vibriosis and more frequently observed in farmed fish. Additionally, heavy parasitic burdens, particularly from *Trichodina* sp. and *C. irritans* (Tables 4 & 5) are likely to compromise epithelial integrity, thereby facilitating bacterial colonization. Lai *et al*. (2023) reported that parasite-induced epithelial damage significantly increases the risk of secondary bacterial infections, including *Vibrio* spp., in marine fish.

In contrast, wild fish displayed lower and more stable *Vibrio* spp. counts throughout the sampling period. Although *Vibrio* spp. present, their abundance remained relatively uniform, and no statistically significant differences were detected among bacterial species. This stability is likely due to better water circulation in open natural environments, lower population densities, and lower nutrient loading from exogenous sources. However, the consistent presence of *Vibrio* spp. suggests that wild populations may act as natural reservoirs, posing biosecurity risks through possible cross-infection, especially in semi-open aquaculture systems lacking proper barriers.

CONCLUSIONS

Based on the results of this study on disease dynamics in barramundi (*L. calcarifer*) in Inner Ambon Bay, it can be concluded that the decline in water quality, particularly low dissolved oxygen (DO) and increased ammonia concentrations at the end of the observation period, is closely associated with the rise in disease prevalence. Clinical symptoms and anatomical changes in organs indicate the presence of complex pathogenic infections. Among bacteria, *V. harveyi* was identified as the dominant and most virulent species, significantly different from *V. alginolyticus* and *V. vulnificus* in farmed fish. Meanwhile, the parasite *Trichodina* sp. showed the highest prevalence and infection intensity, thereby increasing the risk of co-infection with *Vibrio* spp. In addition, wild fish act as pathogen reservoirs, with the potential to transmit diseases to farmed fish populations around floating net cages.

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AUTHOR CONTRIBUTION

RRB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review & editing; JMFS: Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing–original draft, Writing-review & editing; SMAR: Writing–original draft, Writing–review & editing; RHST: Formal Analysis, Funding acquisition, Writing–original draft, Writing–review & editing; JT: Software and Visualization.

DECLARATION OF COMPETING INTEREST

The authors declare no competing interests.

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